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Effects of sludge on germination and initial growth performance of *Leucaena leucocephala* seedlings in the nursery

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Abstract: A study was carried out to determine the influence of different types of sludges (municipal, industrial and residential) on field germination, growth and nodulation of *L. leucocephala* seedlings in the nursery. Before sowing of seeds, different combinations of sludges were incorporated with the nutrient deficient natural forest soils. Field germination, nodulation status and physical growth parameters of seedlings (shoot and root length, vigor index, collar diameter, leaf number, fresh and dry weight of shoot and root and total dry biomass increment) were recorded after three and six months of seed sowing. Field germination, nodulation status and growth parameters were varied significantly in the soil amended with sludges in comparison to control. The highest number of nodule was recorded from soil amended with residential sludge (1:1) and highest fresh and dry nodule weight was also found from the same combination in both three and six month old seedlings. In case of growth parameters, the highest growth was recorded from soil and residential sludge (1:1) combination compared to control. From the study, it can be recommended that soil amended with residential sludge (1:1) provide better field germination, growth and nodule formation of *L. leucocephala* in degraded soil.

Keywords: *Leucaena leucocephala*; Sludge; Field germination; Seedling growth; Nodulation; Vigor index.

Introduction

Ipil ipil [*Leucaena leucocephala* (Lam) de Wit.] is a fast growing multipurpose tree species of the family Leguminosae (Mimosoideae). It is a large evergreen shrub or a small tree depending on its variety and the habitat in which it grows. The tree is native to Mexico and northern Central America and introduced to Indonesia, Philippines, Malaysia and other countries in South East Asia with varying degree of success (Luna 1996). Bangladesh Forest Department introduced *L. leucocephala* in 1977 from the Philippines for a trial plantation (Das 1985). It can produce nutritious forage, firewood, timber and rich organic fertilizer (Duke 1981). The wood has the potential to become a major source of pulp and paper, roundwood and construction materials (Anonymous 1980). The wood, leaves, twigs have a medicinal value as well as tannin. Due to its multipurpose utility and wide range of ecological amplitudes (especially suitable to Bangladesh Environment) *L. leucocephala* is being planted in different parts of Bangladesh by the government and other public and private sectors in different plantation programs, e.g. Agro-forestry, Community Forestry, Social Forestry, Village, Farm Forestry Program etc. (Khan *et al.* 2002). The species has potentiality in newly formed islands and coastal areas of the country where need immediate plantations for suitability of soils and protecting the country from unexpected flood and other natural calamities (Alam *et al.* 2004).

Sewage sludge, also referred as 'biosolids', is being used in agriculture/cropland as a fertilizer and an organic amendment to improve physical, chemical and biological properties of soil (Singh and Agrawal 2007; Sa´nchez-Monedero *et al.* 2004; Hossain and Miller 1994). The safe disposal of the sewage sludge is one of the major environmental concerns throughout the world (Singh and Agrawal 2007). Land application is commonly used in most municipalities (Selivanovskaya and Latypova 2006). Such application of sludge provides not only a means for sludge disposal but can also improve soil fertility and the physical properties of the soils (Peles *et al*. 1996; Ramachandran and D'Soura 1998; Gardiner *et al*. 1995; Jorba and Andres 2000). Disposal alternatives that have been tried so far include soil application, dumping at sea, landfilling and incineration (Sa´nchez-Monedero *et al*. 2004). The increasing cost of chemical fertilizers has reawakened interest in other sources of nutrients, including residential and industrial sludge. The main advantage of using sewage sludge is the soil enrichment at a lower cost that is possible with inorganic fertilizers (Hossain and Miller 1994). Generally sewage sludge is composed of organic compounds, macronutrients, a wide range of micronutrients, non-essential trace metals, organic micro pollutants and microorganisms (Singh and Agrawal 2007; Kulling 2001). The macronutrients in sewage sludge serve as a good source of plant nutrients and the organic constituents provide beneficial soil conditioning properties (Logan and Harrison 1995). Digested cake of sewage sludge contains dry solids (20–50%), organic matter (50%–70%), N (1.5%–2.5%), P (0.5%–1.8%), K (0.1%–0.3%), Ca (1.6%–2.5%) and Mg (0.1%–0.5%) (Byrom and Bradshaw 2001).

Forested sites are increasingly receiving attention as potential sites for the disposal and biological recycling of both wastewater and sludges. These sites are potential for sludge disposal because

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forests are typically located in the better drained areas and not subject to the periodic flooding occurring in alluvial agricultural lands. Moreover, as forests are not food chain crops, many of the public health concerns and land application regulations should not be as critical as those associated with agricultural sites (Cole *et al*. 1983). Fast growing tree species can be benefited from sludge application (Labrecque *et al.* 2006). Though much research has been done on the use of sewage sludge as crop fertilizers (Van den Berg 1993; Hue 1995; Merzlaya *et al.* 1995; Gardiner *et al.* 1995; Selivanovskaya *et al.* 1997; Selivanovskaya and Latypova 1999; Selivanovskaya and Latypova 2006), there is little study in case of *L. leucocephala* especially in soil conditions of Bangladesh. Thus this study is an attempt to find out the effect of sludges on field germination and growth performance as well as nodulation status of *L. leucocephala* seedlings.

Materials and methods

The experiment was carried out in the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong, Bangladesh. The seeds were collected from Bangladesh Forest Research Institute. Degraded soils were collected from hilly sites and were sieved (<3 mm) to obtain a uniform soil size. The brown hilly soils are sandy loam to sandy clay loam, moderately to strongly acid and poorly fertile with pH<5.5, organic matter<2.0%, CEC<10 me/100g and BSP<40% (Osman *et al.* 2001). Sludges were collected from municipal, industrial and residential sites of Chittagong city and dried properly. The dried sludges were also sieved (<3 mm) to make them free from root splinters and other foreign materials. Then the soil and sludges were mixed thoroughly at different ratios. A Complete Randomized Design (CRD) was adopted for a total of seven treatments including a control treatment and three replications for each treatment with 30 polybags for each replication (e.g. 90 polybags for each treatment and a total of 630 polybags for the whole experiment).

There were seven different treatments including the control viz. T_0 (soil, treated as control), T_1 (soil + municipal sludge = 1:1), T_2 (soil + municipal sludge = 2:1), T_3 (soil + industrial sludge = 1:1), T_4 (soil + industrial sludge = 2:1), T_5 (soil + residential sludge = 1:1) and T_6 (soil + residential sludge = 2:1). Polybags (15 cm ×10 cm in size) were filled with the prepared mixtures as mentioned above. Two seeds were sown in an individual polybag to observe the influence of sludge on field germination (e.g. a total of 180 seeds per treatment and 1260 seeds for whole experiment for field germination test) and after completion of field germination only one seedling (best one) per polybag was maintained to observe the initial growth parameters of seedlings. Partial shade and covering was provided over the nursery to protect the seedlings from strong sunlight and rain. Proper care and maintenance were done from the starting time of sowing seed up to harvesting of seedlings. Watering, removal of weeds, grasses etc. were done regularly.

Field germination was recorded daily from the date of seed sown and continued up to last field germination of the seed. The seedlings were allowed to grow for six months from the time of seed sowing. After three months, three seedlings from each replication of a treatment were randomly selected for measuring physical parameters of shoot and root length, collar diameter, leaf number, fresh and dry shoot and root weight, nodule number and their fresh and dry weight. For determining the seedlings dry weight, shoot and root were oven dried at 70ºC until the constant weight was obtained. Vigor index and total dry biomass increment (%) were also calculated by using the following formulae:

Vigor index = Germination (%) × Seedling total length

Total dry biomass increment (%) =

$$\frac{\text{Total dry weight of the treatment - Total dry weight of the control treatment}}{\text{Total dry weight of the control treatment}} \times 100$$

The same work had been done for six months old seedlings. All the data were analyzed statistically by using the computer software package SPSS and they were analyzed by DMRT.

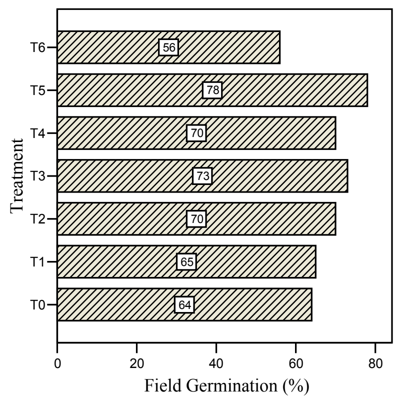

Fig. 1 Effect of sludge on field germination of *L. leucocephala* seeds

Table 1. Effect of sludge on shoot and root length, vigor index, collar diameter and leaf number of 3- and 6- month old *L. leucocephala* seedlings

	Treatment	Length (cm)			Vigor Index	Collar dia. (mm)	Average number of compound leaf
		Shoot	Root	Total			
3-month old seedlings	T_0	17.07 b*	15.72 b	32.79 b	2098 b	1.60 b	7.1 c
	T_1	41.00 a	19.11 ab	60.11 a	3907 a	2.51 a	9.8 b
	T_2	47.44 a	17.50 ab	64.94 a	4546 a	2.62 a	10.2 b
	T_3	46.44 a	24.44 a	70.88a	5174 a	2.96 a	13.4 a
	T_4	44.78 a	20.11 ab	64.89 a	4542 a	2.82 a	10.8 b
	T_5	46.11 a	24.89 a	71.00 a	5538 a	3.12 a	13.8 a
	T_6	42.78 a	21.11 ab	63.89 a	3578 a	2.90 a	10.0 b
6-month old seedlings	T_0	24.67 b	20.00 b	44.67 b	2859 c	2.10 b	11.3 b
	T_1	57.33 a	37.89 a	95.22 a	6189 b	3.63 a	13.1 ab
	T_2	65.11 a	37.89 a	103.00 a	7210 ab	3.82 a	14.1 a
	T_3	67.22 a	39.33 a	106.55 a	7778 a	3.83 a	14.8 a
	T_4	59.22 a	36.00 a	95.22 a	6665 ab	3.35 a	13.7 a
	T_5	71.22 a	43.89 a	115.11 a	8978 ab	3.77 a	12.7 ab
	T_6	64.22 a	40.44 a	104.66 a	5861 ab	3.68 a	12.4 ab

*- Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

Results

Field germination and morphological growth parameters of seedling

The field germination percentage and growth parameters significantly ($P<0.05$) varied in different treatments. The highest field germination (78%) was observed in T_5 and the lowest (56%) in T_6 (Fig. 1). The effects of different sludges on morphological growth parameters of seedlings like shoot length, root length, total length, collar diameter and leaf number of three and six months old seedlings were shown in Table 1. In three months old seedlings, shoot growth was the highest (47.44 cm) in T_2 whereas, the highest root growth (24.89 cm) was recorded in T_5. Collar diameter was highest (3.12 mm) in T_5 and was significantly ($P<0.05$) different from that of the control. Vigor index was highest (5538) in T_5 followed by T_3 and T_2 and was also significantly ($P<0.05$) different from that of T_0. The highest (13.8) average number of compound leaf was recorded in T_5, whereas the lowest (7.1) was in T_0 (control). In case of six months old seedlings, the highest shoot growth (71.22 cm) was also recorded in T_5 and the lowest in T_0. The highest root growth (43.89 cm) was found in T_5. Collar diameter was highest (3.83 mm) in T_3 and was significantly ($P<0.05$) different from that of T_0. Vigor index was highest (8978) in T_5 followed by T_3 and T_2 and was significantly ($P<0.05$) different from that of T_0. The average number of compound leaf was maximum (14.8) in T_3 followed by 14.1 and 13.7 in T_2 and T_4, respectively whereas, the lowest (11.3) was in T_0.

Table 2. Effect of sludge on shoot and root fresh, dry weight and total biomass increment of 3- and 6- month old *L. leucocephala* seedlings

	Treatment	Fresh weight (g)			Dry weight (g)			Total dry biomass increment (%)
		Shoot	Root	Total	Shoot	Root	Total	
	T_0	1.19 c *	0.30 b	1.49 c	0.31 b	0.03 b	0.34 b	00.00
3-month old	T_1	3.87 b	1.02 ab	4.89 b	1.22 ab	0.34 a	1.56 ab	+ 358.82
seedlings	T_2	4.35 b	1.00 ab	5.35 ab	1.27 ab	0.14 ab	1.41 ab	+ 314.70
	T_3	5.42 ab	1.17 a	6.59ab	1.43 ab	0.18 ab	1.61 ab	+ 373.52
	T_4	5.01 ab	0.97 ab	5.98 ab	1.24 ab	0.17 ab	1.41 ab	+ 314.70
	T_5	7.31 a	1.33 a	8.64 a	1.90 a	0.29 a	2.19 a	+ 544.11
	T_6	5.05 ab	0.87 ab	5.92 ab	1.55 a	0.21 ab	1.76 a	+ 417.64
	T_0	2.14 b	0.69 b	2.83 b	0.61 b	0.22 b	0.83 b	00.00
6-month old	T_1	9.50 a	2.00 ab	11.50 ab	3.48 a	0.78 ab	4.26 a	+ 413.25
seedlings	T_2	10.72 a	2.87 a	13.59 a	3.82 a	1.08 a	4.90 a	+ 490.36
	T_3	13.18 a	2.84 a	16.02 a	4.76 a	1.06 a	5.82 a	+ 601.20
	T_4	9.03 ab	2.42 ab	11.45ab	3.06 a	0.91 ab	3.97 a	+ 378.31
	T_5	13.27 a	3.33 a	16.60 a	4.62 a	1.26 a	5.88 a	+ 608.43
	T_6	10.76 a	2.30 ab	13.06 a	4.00 a	0.89 ab	4.89 a	+ 489.16

*- Means followed by the same letter (s) in the same column do not vary significantly at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

Table 3. Effect of sludge on nodule number and fresh and dry weights of 3- and 6- month old L. *leucocephala* seedlings

	Treatment	Nodule					
		Number	Weight (g)		Number increased/ decreased (%)	Weight increased/ decreased (%)	
			Fresh	Dry		Fresh	Dry
	T_0	1 d *	0.020 c	0.004 d	00.00	00.00	00.00
3-month old	T_1	6 ab	0.057 ab	0.021 bc	+ 500.00	+ 185.00	+ 425.00
seedlings	T_2	5 bc	0.052 b	0.020 bc	+ 400.00	+ 160.00	+ 400.00
	T_3	6 ab	0.066 ab	0.026 ab	+ 500.00	+ 230.00	+ 550.00
	T_4	5 bc	0.055 b	0.020 bc	+ 400.00	+ 175.00	+ 400.00
	T_5	7 a	0.075 a	0.032 a	+ 600.00	+ 275.00	+ 700.00
	T_6	4 c	0.053 b	0.014 c	+ 300.00	+ 165.00	+ 250.00
	T_0	4 d	0.077 bc	0.045 b	00.00	00.00	00.00
6-month old	T_1	15 c	0.136 a	0.075 a	+ 275.00	+ 76.62	+ 66.66
seedlings	T_2	14 c	0.115 ab	0.054 b	+ 250.00	+ 49.35	+ 20.00
	T_3	19 b	0.136 a	0.052 b	+ 375.00	+ 76.62	+ 15.55
	T_4	14 c	0.075 bc	0.018 c	+ 250.00	- 2.59	- 60.00
	T_5	24 a	0.095 bc	0.033 bc	+ 500.00	+ 23.37	- 26.66
	T_6	16 bc	0.068 c	0.020 c	+ 300.00	- 11.68	- 55.55

*- Means followed by the same letter (s) in the same column do not vary significantly at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

Fresh and dry matter production

Fresh and dry matter production, e.g. shoot and root fresh weight, total fresh weight, shoot and root dry weight and total dry weight were shown in Table 2. In three months old seedlings, both fresh and dry shoot weights were maximum (7.31 g and 1.90 g, respectively) in T_5 and were significantly ($P<0.05$) different from those of T_0. Fresh root weight was maximum (1.33 g) in T_5 whereas, the highest (0.34 g) root dry weight was found in T_1. In all the cases the lowest growth was observed in T_0 (Table 2). Total dry biomass increment (%) was highest in T_5 followed by T_6 and T_3 and was positive for all the treatments compared to T_0. In case of six months old seedlings, fresh shoot weight was maximum (13.27 g) in T_5, whereas, dry shoot weight was the

highest (4.76g) in T_3 and significantly (P<0.05) different from those of T_0. Both fresh and dry root weights were maximum (3.33 g and 1.26 g, respectively) in T_5 and were significantly (P<0.05) different from those of T_4, T_6, T_1 and T_0. In all the cases the lowest growth was observed in T_0 (Table 2). Total dry biomass increment (%) was highest in T_5 followed by T_3 and T_2 and was positive for all the treatments compared to T_0 treatment.

Nodulation status of seedling

Nodulation status of seedlings is presented in Table 3. In three months old seedlings, the highest (7) nodule number was found in T_5 and the lowest in T_0. Both fresh and dry weights of nodules were maximum (0.075 g and 0.032 g, respectively) in T_5 and lowest (0.020 g and 0.004 g, respectively) in T_0. The rate of nodule number increment was positive in all the treatments as compare to T_0. Nodule fresh and dry weight increment were also found positive in all the treatments compare to T_0. In case of six months old seedlings, nodule number was highest (24) in T_5 and the lowest (4) in T_0. Fresh weight of nodule was maximum (0.136 g) both in T_1 and T_3. Dry weights of nodules were maximum (0.075 g) in T_1. Both fresh and dry weights of nodules were lowest (0.068 g and 0.020 g, respectively) in T_6. The rate of nodule number increment was positive in all the treatments compare to T_0. The increment rate of nodule fresh weight was positive in T_1, T_2, T_3 and T_5 and negative for T_4 and T_6. The increment rate of nodule dry weight was positive in T_1, T_2 and T_3 and negative for T_4, T_5 and T_6 in comparison with T_0 treatment.

Discussion

The present study indicates that the field germination percentage, growth parameters (shoot and root length, vigor index, collar diameter, leaf number, fresh and dry weight of shoot and root and total dry biomass increment) and nodule formation of seedlings recorded from different combinations of sludge treatments in *L. leucocephala* varied significantly compared to control. The present findings are in agreement with Jorba and Andres (2000) that sewage sludge could serve as a good organic fertilizer to maximize plant germination. Sludge amendments enhanced the germination and decreased the mortality of the seedlings. The effects were more obvious for the soil with the highest sludge treatment. The beneficial effects on the biomass of seedlings and the height of the shoots as well as on the length of the roots of the pine seedlings were greater in plots with the highest rates of composted sludge (Selivanovskaya and Latypova 2006). The present study was also coincided with Labrecque *et al*. (2006) who reported that fast growing species can get benefit from sludge amended soil. Sludge typically contains large amounts of plants available N and P, smaller amounts of all the secondary and micronutrients and in addition, supplies organic matters (Bates *et al*. 1979). The nitrogen content in the sludge is usually released more slowly than commercial fertilizers (Riha *et al*. 1983). This slower release is probably more appropriate for tree growths. Unfortunately, sewage sludge may also contain a range of potentially toxic metals, such as Cu, Cd, Pb, Zn and Ni (Logan and Chaney 1983). The simultaneous phyto-separation of toxic and beneficial elements from sewage sludge are possible by co-cropping using specific plants without the input of any chemicals (Wu *et al*. 2007). Khan *et al*. (2002) also noticed positive growth on *L. leucocephala* by using beneficial microbial inoculants (Effective Microorganisms) in different combinations. In the present study almost all growth parameters especially field germination (%), shoot length (cm) and shoot fresh weight (g) were found better than that findings. The parameters (total length, collar diameter and number of compound leaf) of *L. leucocephala* were better than that reported by Alam *et al*. (2004) where they used three types of media viz. peat soil + cowdung (3:1), soil + cowdung (3:1) and soil + peat soil (1:1) in three types of containers viz. transparent polybag (10 cm × 15 cm), plastic seed-tray (24.5 cm dia.) and open seed bed. Thus the influence of sludge amended soil on plants is positive which is comparable to findings recorded by others (Jorba and Andres 2000; Labrecque *et al*. 2006; Selivanovskaya and Latypova 2006).

Conclusion

From the present findings it may be concluded that residential (soil + residential sludge = 1:1) sludge may be used for obtaining maximum seed field germination and optimum seedling growth and nodule formation of *L. leucocephala* and helps to obtain healthy seedlings that can be easily established in degraded sites. Results from sludge fertilization experiments in forests in many parts of the world showed that most tree species respond positively to sludge fertilization. Residential sludges provide a potential source of nutrients to the forest crops especially fast growing species and such use can satisfy the needs for environmentally safe disposal of sludge.

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